

## CLAIMS

- 1    1. A method for detecting an unbound form of a first member of a binding pair, the  
2        binding pair comprising a first and second member, each member bindable to the  
3        other, the method comprising the steps of:
  - 4            (a) providing a first particle bound to the second member;
  - 5                    (b) reacting the first particle bound to the second  
6        member with a sample, thereby forming a first complex between  
7        the second member bound to the first particle and unbound first  
8        member present in said sample;
  - 9            (c) providing a second particle bound to a third member, the third  
10      member being different from the second member and being  
11      capable of binding to the first member;
  - 12                    (d) reacting the second particle bound to the third  
13      member to the sample, thereby forming a second complex between  
14      the third member bound to the second particle and the first  
15      complex; and
  - 16                    (e) detecting any second complex formed.
- 1    2. The method of claim 1, wherein the third member is an antibody which  
2        specifically binds to the first member.
- 1    3. The method of claim 1, wherein the first and/or second particle is latex.
- 1    4. The method of claim 1, wherein the second complex is detected by measuring an  
2        increase in the turbidity of the sample.
- 1    5. The method of claim 1, wherein steps (a) through (d) are performed sequentially.
- 1    6. The method of claim 1, wherein steps (a) through (d) are performed  
2        simultaneously.
- 1    7. The method of claim 1, wherein the amount of second complex formed is  
2        quantitated.
- 1    8. The method of claim 1, wherein the first member is protein S.
- 1    9. The method of claim 1, wherein the second member is C4b-binding protein  
2        (C4BP).

- 1    10. The method of claim 1, wherein the sample is selected from the group consisting  
2        of blood, plasma, serum, saliva, CSF, urine, culture media, a cell suspension, a  
3        buffer and an artificially prepared fluid containing the first member.
- 1    11. The method of claim 1, wherein the second member binds to the first member at a  
2        single binding site.
- 1    12. The method of claim 11, wherein the third member binds to the first member at a  
2        single binding site which is different from the single binding site to which the  
3        second member binds.
- 1    13. The method of claim 1, wherein step (b) is performed within 0 to about 180  
2        seconds.
- 1    14. The method of claim 1, wherein the molar ratio of third member to second  
2        member is between about 2 and 20.
- 1    15. The method of claim 1, wherein the molar ratio of the third member to second  
2        member is between about 5 and 10.
- 1    16. The method of claim 1, wherein the amount of third member is higher than the  
2        amount of free first member in the sample.
- 1    17. The method of claim 1, wherein the molar ratio of third member is between about  
2        10 and 40 times the amount of free first member in the sample.
- 1    18. A composition for detecting an unbound form of a first member of a binding pair,  
2        the binding pair comprising a first and second member, each member bindable to the  
3        other,      the composition comprising:  
4                  a first particle bound to the second member;  
5                  a second particle bound to a third member, the third member being  
6                  different from the second member and capable of binding to the first  
7                  member at a binding site different from the second member.
- 1    19. The composition of claim 18, wherein the first member is protein S and the  
2        second member is C4BP.
- 1    20. The composition of claim 18, wherein the third member is an antibody and the  
2        second member is not an antibody.
- 1    21. The composition of claim 18, wherein the second member comprises a single  
2        binding site for the first member.

- 3       22. The composition of claim 21, wherein the third member binds to the first member  
4                  at a single binding site which is different from the single binding site to which the  
5                  second member binds.
- 1       23. A method for detecting an unbound form of a first member of a binding pair, the  
2                  binding pair comprising a first and second member, each member bindable to the  
3                  other, the method comprising the steps of:
- 4                      (a) providing a first particle bound to the second member;  
5                      (b) reacting the first particle bound to the second member with a  
6                          sample, thereby forming a first complex between the second  
7                          member bound to the first particle and unbound first member  
8                          present in said sample;
- 9                      (c) providing a second particle bound to the first member;  
10                     (d) reacting the second particle bound to the first member with the  
11                          sample, thereby forming a second complex between second  
12                          particle bound to the first member and first particle bound to  
13                          second member which is not already bound to the first member;  
14                          and
- 15                     (e) detecting any second complex formed, wherein the amount of  
16                          second complex formed is inversely proportional to the amount of  
17                          unbound first member in the sample.
- 1       24. The method of claim 23, wherein the first and/or second particle is latex.
- 1       25. The method of claim 23, wherein the second complex is detected by measuring an  
2                  increase in the turbidity of the sample.
- 1       26. The method of claim 23, wherein the amount of second complex formed is  
2                  quantitated.
- 1       27. The method of claim 23, wherein the first member is protein S.
- 1       28. The method of claim 23, wherein the second member is C4BP.
- 1       29. The method of claim 23, wherein the sample is selected from the group consisting  
2                  of blood, plasma, serum, or an artificially prepared buffer containing the first  
3                  member.

- 1 30. A composition for detecting an unbound form of a first member of a binding pair  
2 comprising a first and second member, each member bindable to the other, the  
3 composition comprising:  
4           a first particle bound to the second member; and  
5           a second particle bound to the first member.

1 31. The composition of claim 30, wherein the first member is protein S and the  
2 second member is C4BP.

1 32. A method for diagnosing thrombophilia comprising performing the method of  
2 claim 8, and further comprising comparing the amount of second complex formed  
3 to the amount of second complex formed in a sample derived from an individual  
4 without thrombophilia.

1 33. A method for diagnosing thrombophilia comprising performing the method of  
2 claim 27, and further comprising comparing the amount of second complex  
3 formed to the amount of second complex formed in a sample derived from an  
4 individual without thrombophilia.